JOURNAL OF CLINICAL MICROBIOLOGY, Apr. 1985, p. 626–629
0095-1137/85/040626-04$02.00/0

Vol. 21, No. 4

Controlled Evaluation of Trypticase Soy Broth in Agar Slide and Conventional Blood Culture Systems

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Received 26 September 1984/Accepted 24 December 1984

A commercially available biphasic blood culture system that utilizes an attachable agar slide paddle and Trypticase soy broth (BBL Microbiology Systems, Cockeysville, Md.) was compared with a conventional Trypticase soy broth blood culture bottle in 6,867 paired blood cultures from adult patients. Both systems were inoculated with equal volumes of blood (5 ml) and incubated aerobically (vented) for 2 weeks. More clinically important bacteria and fungi, including *Staphylococcus epidermidis*, streptococci, *Escherichia coli*, *Klebsiella* spp., other *Enterobacteriaceae*, and *Pseudomonas aeruginosa*, were recovered from the biphasic system ($P < 0.001$). In contrast, more anaerobic bacteria of importance were recovered in the conventional bottle ($P < 0.01$). Staphylococci ($P < 0.001$), gram-negative facultative and aerobic bacteria ($P < 0.001$), and fungi ($P < 0.001$) were detected 1 or more days earlier in the biphasic system, whereas pneumococci ($P < 0.05$) were detected earlier in the conventional bottle. Of 603 clinically important microorganisms that grew in the biphasic system, 601 (99.7%) were detected by day 7 of incubation, but only 403 of 490 microorganisms (82.2%) were detected by day 7 in the conventional bottle. Overall, the biphasic system was superior to the conventional bottle. For optimal detection of anaerobic bacteremia, however, the biphasic system should be used in conjunction with a complementary anaerobic conventional bottle.

In addition to conventional broth blood culture systems, clinical microbiology laboratories now have the opportunity to select other commercially available, innovative systems for processing blood for culture. These include radiometric testing, lysis and centrifugation, and a recently marketed biphasic system that utilizes an attachable agar slide paddle for performing repeated subcultures. An earlier report of 2,662 blood cultures showed that the agar slide blood culture system had an increased yield and speed of detection compared with a conventional system (3). The present evaluation of 6,867 blood cultures at three collaborating hospitals was undertaken to confirm and extend these observations.

MATERIALS AND METHODS

Collection of samples. During the study period, a 45-ml bottle of Trypticase soy broth (TSB; BBL Microbiology Systems, Cockeysville, Md.) with 0.03% sodium polyanethol sulfonate and a 70-ml bottle of TSB with 0.05% sodium polyanethol sulfonate were used for all blood cultures from adult patients at Middlesex General-University Hospital, the University of Colorado Hospital, and the Denver Veterans Administration Medical Center. Patient blood cultures were obtained at the bedside after preparation of the skin with 10% povidone–iodine (1% available iodine) followed by 70% isopropyl alcohol. Blood from each separate venipuncture was distributed by needle and syringe as follows: 5 ml was inoculated into the bottle with 45 ml of TSB (CB; Becton Dickinson Vacutainer Systems, Rutherford, N.J.), and 5 ml of blood was inoculated into the bottle with 70 ml of TSB (RSC; Roche Diagnostics Systems, Nutley, N.J.). Thus, the volume of blood was the same for both culture bottles (5), although the ratio of blood to broth was not the same (1:9 and 1:14, respectively). Both blood culture bottles had been evacuated and back-flushed with 10% carbon dioxide in nitrogen at stoppering during the manufacturing process.

Volume standards. To ensure that the culture bottles actually received the specified amounts of blood, we measured the level of fluid in each container after it was filled with blood. Although all blood-containing bottles were incubated, those with fluid levels below or above the standards were coded as inadequate and were excluded from subsequent analyses. Fluid level standards were set to ensure that at least 4 ml, but no more than 6 ml, of blood was added to each bottle.

Processing of samples. Identical methods were used for processing the blood cultures in the clinical microbiology laboratories at all three hospitals. Both bottles were incubated aerobically at 35°C for 14 days. When the paired samples were received, a sterile open venting unit that allowed continuous exchange of air was placed on the CB (6), and an agar slide paddle was attached to the RSC. Cultures were examined macroscopically twice during the first 24 h and daily thereafter for 7 days; they were then reincubated until the final subcultures were done on day 14 of incubation.

In the CB, subcultures were done through the needle of the open venting unit after 1 and 14 days of incubation. On day 1 aerobic subculture was done on chocolate agar incubated in 5% CO_2 at 35°C. In addition, acridine orange stain was performed after 12 to 24 h of incubation (2) on all macroscopically negative CB bottles. On day 14 aerobic (chocolate agar) and anaerobic (5% sheep blood agar or brucella agar with vitamin K_1 and hemin) subcultures were done.

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After attachment of the slide paddle, RSC was immediately subcultured by inverting the bottle and allowing the blood-broth mixture to cover the agar-coated paddle. This process was repeated after 4 to 8 h of incubation, at least once daily through day 7, and again on day 14. The paddle and the broth were examined daily for evidence of growth. If a bottle was positive macroscopically in the broth, but no growth was evident on the agar paddles, the slide chamber was removed, and a sterile Pasteur pipette was used to obtain broth for Gram stain and subcultures. If growth on the agar paddle was noted at the same time the broth became positive macroscopically, the initial mode of detection was considered to be the growth on agar (i.e., subculture).

Clinical assessment. All patients with positive blood cultures were evaluated by an infectious disease specialist who defined pathogens (clinically important bacteria causing sepsis) and contaminants by using established criteria (9).

Analysis of data. Paired comparisons of the two blood culture bottles were done only on adequately filled (≥4 ml and ≤6 ml of blood) bottles that grew microorganisms causing true bacteremia or fungemia. Significance testing was done with the modified chi-square test described by McNemar (1).

RESULTS

A total of 6,867 adequately filled blood culture sets were received during the study period. Of these, 951 (13.8%) were positive, including 608 (8.9%) that grew microorganisms causing illness, 269 (3.9%) that grew contaminants, 13 (0.2%) that grew one or more contaminants and pathogens, and 61 (0.9%) that grew organisms that were indeterminate as a cause of sepsis. A total of 682 isolates associated with sepsis were detected; 411 isolates grew in both systems, and 209 of these were detected on the same day. A total of 606 (88.9%) isolates associated with sepsis grew in RSC, whereas 491 (72.0%) grew in CB.

Overall, clinically important bacteria were recovered more often ($P < 0.001$) in the agar slide system (Table 1). This improved yield could be attributed to better detection of both gram-positive and gram-negative aerobic and facultative bacteria, specifically, *Staphylococcus epidermidis* ($P < 0.001$), streptococci ($P < 0.001$), *Escherichia coli* ($P < 0.01$), *Klebsiella* spp. ($P < 0.01$), other *Enterobacteriaceae* ($P < 0.01$), and *Pseudomonas aeruginosa* ($P < 0.02$). Although not statistically significant, both *Staphylococcus aureus* and fungi were favored by the agar slide system. In contrast, recovery of anaerobic bacteria was favored by the conventional system ($P < 0.01$).

Dramatic differences were noted in the speed with which the two systems detected positive cultures (Table 2). Microorganisms detected earlier in the agar slide system included gram-positive facultative and aerobic bacteria ($P < 0.02$), particularly staphylococci ($P < 0.001$), gram-negative facultative and aerobic bacteria ($P < 0.001$), and fungi ($P < 0.001$). In contrast, pneumococci were detected earlier in the conventionally processed TSB ($P < 0.05$).

Contaminant isolates were detected with greater frequency in the agar slide system than in the conventional system (Table 3). In particular, *S. epidermidis* ($P < 0.001$) and *Corynebacterium* spp. ($P < 0.001$) were detected more frequently in the agar slide system. Overall, 98 contaminant isolates were detected in the conventional system, and 240 isolates were detected in the agar slide system.

Since many laboratories routinely hold blood cultures for 7 rather than 14 days, as was done in this study, we analyzed the data to determine what proportion of isolates might be missed by each system if incubation were limited to 7 days. In the agar slide system, only 2 of 603 isolates (1 *S. aureus*, 1 *Candida albicans*) would have been missed. In the CB, 83 of 491 isolates (16.9%), including 13 *S. aureus*, 8 streptococci, 9 *E. coli*, 13 other *Enterobacteriaceae*, and 27 *Candida* spp., were not detected after 7 days of incubation. However, since subcultures of the CB were not done at 7 days, the number of isolates missed probably is overestimated in this analysis.

TABLE 1. Comparison of yield of clinically important bacteria and fungi from 5-ml samples of blood cultured in CB and RSC

Microorganisms	No. of isolates from:			P
	Both CB and RSC	CB only	RSC only	
Aerobic and facultative bacteria	340	48	167	<0.001
Gram-positive	139	18	70	<0.001
Staphylococcus aureus	78	6	16	NS[a]
Staphylococcus epidermidis	4	2	18	<0.001
Streptococcus pneumoniae	21	4	3	NS
Viridans streptococci	8	3	13	<0.05
Other streptococci[b]	24	3	18	<0.01
Other[c]	4	0	2	NS
Gram-negative	201	30	97	<0.001
Escherichia coli	65	11	29	<0.01
Klebsiella spp.	25	3	15	<0.01
Other *Enterobacteriaceae*	70	8	27	<0.01
Pseudomonas aeruginosa	28	8	22	<0.02
Other[d]	13	0	4	NS
Anaerobic bacteria	12	24	8	<0.01
Gram positive[e]	1	12	2	<0.02
Gram negative[f]	11	12	6	NS
All bacteria	352	72	175	<0.001
Fungi[g]	59	7	17	NS

[a] NS, Not significant ($P > 0.05$).
[b] Six group A streptococci, 7 group B streptococci, 20 enterococci, and 12 other streptococci.
[c] Two *Gardnerella vaginalis* and four *Listeria monocytogenes*.
[d] One *Eikenella* sp., six *Acinetobacter* spp, one *Pseudomonas* sp., four *Cardiobacterium* spp., and five *Haemophilus influenzae*.
[e] Four *Clostridium* spp., one *Bifidobacterium* sp., one *Eubacterium* sp., two *Propionibacterium* spp., two peptostreptococci, four peptococci, and an unidentified gram-positive rod.
[f] Twenty-seven *Bacteroides* spp., one *Fusobacterium* sp., and one *Veillonella* sp.
[g] Forty-six *Candida albicans*, 26 *Candida tropicalis*, 9 *Candida parapsilosis*, 1 *Candida guillermondi*, and 1 *Torulopsis glabrata*.

DISCUSSION

This multihospital evaluation of the agar slide blood culture system has demonstrated it to be an excellent means of detecting aerobic and facultative bacteria and fungi, confirming and expanding the results of an earlier study (3). As in the report by Pfaller et al. (3), gram-positive bacteria and fungi were detected more frequently in the agar slide system than in TSB processed conventionally. In this evaluation, significantly more *Enterobacteriaceae*, especially *E. coli* and *Klebsiella* spp., and *P. aeruginosa* were detected in the agar slide system. However, the agar slide system detected significantly fewer anaerobic bacteria, particularly gram-positive anaerobes, and for this reason cannot be considered an ideal single-bottle blood culture system.

The high mortality of bacteremia (8) mandates early intervention with specific therapy in an attempt to improve outcome, and the early detection of the etiological microorganisms is an important part of this process. Therefore, we analyzed the speed with which agar slide and conventional blood culture bottles detected growth when both were positive. Although earlier detection in the agar slide system was suggested by Pfaller et al. (3), formal analysis was not presented. The findings in this study (Table 2) revealed a significant speed advantage in the agar slide system for both aerobic and facultative bacteria and fungi, in all probability related to the ease with which frequent subcultures can be performed and perhaps the enhanced oxygenation during the subculture process.

Although 7-day incubation of blood cultures is appropriate in many settings (4), we have noted incubation for 2 weeks to be superior when conventional blood culture systems are used (7). Longer incubation, however, requires increased space requirements as well as more technologist time. The impressive yield after 7 days of incubation in the agar slide bottle suggests that longer holding times are not necessary in laboratories using this system.

The increased contamination rate in the agar slide system has been noted by others (3). The somewhat higher rate in our study (3.5%) compared with that of Pfaller et al. (2.7%) may have been due to methodological differences (3). In our study, all handling of the blood culture bottles and agar slide paddles was done at the bench, whereas in the study by Pfaller et al. the initial attachment of the paddle was done under a laminar air flow hood. We noted that examination of the agar paddles sometimes was difficult due to condensation on the inner surface of the clear plastic cylinder holding the paddle. This required the technologists to remove the paddle to examine the agar surfaces completely, and the additional manipulation may have contributed to an increased contamination rate. It is possible that performing all manipulations under a laminar air flow hood would result in decreased rates of contamination with this system.

In conclusion, the agar slide blood culture system detected more organisms causing sepsis than did the conventional system. When both systems detected bacteremia, the agar slide system did so significantly earlier than the conventional system. Of 603 clinically important isolates, 601 (99.7%) detected in the agar slide system were found by day 7 of incubation, whereas only 403 of 490 pathogens (82.2%) were detected by day 7 in the conventional system. The agar slide system detected approximately twice the number of contaminant isolates as the conventional system and significantly fewer anaerobes. For optimal detection of anaerobic bacteremia, therefore, the aerobic agar slide blood culture system should be used in conjunction with an anaerobic bottle.

TABLE 2. Comparison of speed of detection of clinically important bacteria and fungi grown from both 5-ml samples of blood cultured in CB and RSC[a]

Microorganisms	No. of isolates from:			P
	CB and RSC (same time)	CB ≥1 day earlier	RSC ≥1 day earlier	
Aerobic and facultative bacteria	193	47	99	<0.001
Gram positive	86	17	36	<0.02
Staphylococci	47	3	32	<0.001
S. pneumoniae	15	6	0	<0.05
Viridans streptococci	4	3	1	NS
Other streptococci	17	4	3	NS
Other	3	1	0	NS
Gram negative	107	30	63	<0.001
E. coli	30	13	22	NS
Serratia marcescens	19	6	17	<0.05
Other *Enterobacteriaceae*	37	5	11	NS
P. aeruginosa	16	5	7	NS
Other	5	1	6	NS
Anaerobic bacteria	4	3	5	NS
Gram positive	1	0	0	NS
Gram negative	3	3	5	NS
All bacteria	197	50	104	<0.001
Fungi	12	1	46	<0.001

[a] See footnotes *a* through *g* of Table 1.

TABLE 3. Comparison of yield of contaminant bacteria and fungi from 5-ml samples of blood cultured in CB and RSC

Microorganisms[a]	No. of isolates from:			P
	Both CB and RSC	CB only	RSC only	
S. epidermidis	35	29	123	<0.001
Bacillus spp.	1	2	8	NS[a]
Corynebacterium spp.	4	6	37	<0.001
Propionibacterium spp.	1	9	15	NS
Other[b]	2	9	14	NS
All contaminants	43	55	197	<0.001

[a] NS, Not significant ($P > 0.05$).

[b] Four *S. aureus*, six viridans streptococci, three micrococci, two *Neisseria* spp., two *Clostridium* spp., and one each of enterococci, *Gardnerella* sp. *Klebsiella* sp., *Pseudomonas* sp., *Haemophilus* sp., *Eubacterium* sp., *Peptococcus* sp., and *C. tropicalis*.

ACKNOWLEDGMENT

This work was supported in part by a grant from Roche Diagnostic Systems, Nutley, N.J.

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